

IX. ABSTRACT

Beta-amyloid peptide (β A) is a major fibrillar component of neuritic plaques in Alzheimer's disease brains and is related to the pathogenesis of the disease. β A generation depends on proteolytic cleavage of the amyloid precursor protein (APP).

5 The present invention is a new procedure for the cloning of human β A precursor protein gene (human APP gene) based on the reverse transcription (RT) and the polymerase chain reaction (PCR). This procedure for cloning human APP gene by means of RT-PCR reactions is cost-effective, not time-consuming, and is suited for any laboratory.

10 The present invention also includes a new procedure for the construction of expression plasmids, a/ using the pFastBacTM HTb and the pBlueBacHis2 A transfer vectors for the purpose of obtaining human APP in insect cells; and b/ using the pET-28a (+) transfer vector for the purpose of obtaining human APP in bacteria.

15 The present invention makes it easier to obtain full-length APP which is essential for the identification of biological activities that occur in the APP molecule and for the identification of proteases capable of creating β A. Knowing which protease creates β A is important for the exploration of therapeutic and preventative strategies for the treatment of Alzheimer's disease.

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LEGEND OF FIGURES

Figure 1: PCR amplification of the human APP gene.

M: Marker.

1: PCR product of the human APP gene (~ 2.3 kb).

5 **Figure 2:** Cloning of the APP-cDNA into pCR^R II plasmid vector.

The arrow head point is the direction of transcription.

(1): pCR^R II/APP₇₅₁-cDNA.

(2): pCR^R II/APP₇₇₀-cDNA.

Figure 3: Construction of pFastBacTM HTb/APP-cDNA.

10 The arrow head point is the direction of transcription.

(3): pFastBacTM HTb/APP₇₅₁-cDNA.

(4): pFastBacTM HTb/APP₇₇₀-cDNA.

(5): APP₇₅₁-cDNA in bacmid of DH10BacTM E. Coli.

(6): APP₇₇₀-cDNA in bacmid of DH10BacTM E. Coli.

15 **Figure 4:** Construction of pBlueBacHis2 A/APP-cDNA.

The arrow head point is the direction of transcription.

(7): pBlueBacHis2 A/APP₇₅₁-cDNA.

(8): pBlueBacHis2A/APP₇₇₀-cDNA.

Figure 5: Construction of pET-28a (+)/APP-cDNA.

20 The arrow head point is the direction of transcription.

(9): pET-28a (+)/APP₇₅₁-cDNA.

(10): pET-28 (+)/APP₇₇₀-cDNA.